

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification⁵ : A61K 37/30, 47/26, 47/10 A61K 9/00	A1	(11) International Publication Number: WO 93/06854 (43) International Publication Date: 15 April 1993 (15.04.93)
(21) International Application Number: PCT/EP92/02321 (22) International Filing Date: 8 October 1992 (08.10.92) (30) Priority data: MI91A002703 11 October 1991 (11.10.91) IT (71) Applicant (for all designated States except US): SMITH-KLINE BEECHAM FARMACEUTICI S.P.A. [IT/IT]; Via Zambelletti, I-20021 Baranzate di Bollate (IT). (72) Inventors; and (75) Inventors/Applicants (for US only): CHIODINI, Laura [IT/IT]; FONIO, Teodoro [IT/IT]; PINZA, Mario [IT/IT]; Smithkline Beecham Farmaceutici S.p.A., Via Zambelletti, I-20021 Baranzate di Bollate (IT).		(74) Agent: FLORENCE, Julia, A.; Corporate Patents, Smith-Kline Beecham, Mundells, Welwyn Garden City, Hertfordshire AL7 1EY (GB). (81) Designated States: AU, BR, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING A CALCITONIN, A GLYCYRRHIZINATE AS ABSORPTION ENHANCER AND BENZYL (57) Abstract Pharmaceutical compositions comprising a calcitonin, an effective amount of an absorption enhancer which is a glycyrrhizinate, an effective amount of benzyl alcohol and a pharmaceutically acceptable carrier are useful in the treatment of conditions such as osteoporosis.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	LI	Liechtenstein	SK	Slovak Republic
CI	Côte d'Ivoire	LK	Sri Lanka	SN	Senegal
CM	Cameroon	LU	Luxembourg	SU	Soviet Union
CS	Czechoslovakia	MC	Monaco	TD	Chad
CZ	Czech Republic	MG	Madagascar	TC	Togo
DE	Germany	ML	Mali	UA	Ukraine
DK	Denmark	MN	Mongolia	US	United States of America
ES	Spain			VN	Viet Nam
FI	Finland				

- 1 -

PHARMACEUTICAL COMPOSITIONS COMPRISING A CALCITONIN, A GLYCYRRHIZINATE AS ABSORPTION ENHANCER AND BENZYL

The present invention relates to novel pharmaceutical compositions containing calcitonins, and to a novel method of enhancing the absorption of a calcitonin across a mucosal membrane.

The calcitonins are a class of pharmacologically active peptides, of both natural and synthetic origin, which contain approximately thirty two amino acids, and which have the ability to regulate serum calcium levels.

Various calcitonins, including e.g. natural human, salmon and eel calcitonins and the synthetic eel calcitonin analogue elcatonin are now commercially available and commonly employed, e.g. in the treatment of Paget's disease, Sudeck's disease and osteoporosis.

A considerable and well known problem with the administration of peptides is that they are susceptible to rapid acid and enzyme-induced degradation when administered orally. For this reason, parenteral administration has been, hitherto, the most widely used means of administration and, in the case of peptides of higher molecular weight, such as the calcitonins, has been the only significant effective means of administration.

It is widely recognised that administration by injection can be both inconvenient and unpleasant for the patient, particularly when the administration has to be repeated at regular intervals for long periods, e.g. in the treatment of post-menopausal osteoporosis with calcitonins. Thus, there has been growing interest in the administration of peptides by more acceptable non-invasive alternative routes, for example in the form of sublingual tablets, suppositories, intrapulmonary powders, intranasal drops, sprays, powders, gels, ointments and inserts.

A significant problem with many peptides, particularly those of higher molecular weights, is that they are only poorly absorbed across biological membranes, e.g. mucosal membranes, and thus the bioavailability of the peptide is often very low. Considerable research has therefore been carried out in order to find methods of improving the trans-epithelial absorption of peptides. One approach is to use an adjuvant or absorption enhancer and there are numerous published reports of compounds which are claimed to have peptide absorption-enhancing properties.

Thus, for example, choline esters (EP 214898), acyl carnitines (EP 215697), aldoses and glucosamines (Japanese Pat. Appl. No. 61 126034), ascorbates and salicylates (EP 37943), alpha-cyclodextrin (EP 0094157), pyroglutamate esters (EP 173990), chelating agents (US 4,476,116) ethanol, benzyl alcohol and polyethylene glycol 400 (EP 371010) have been proposed as absorption enhancers.

There are many published reports that surfactants can enhance the absorption of polypeptides, see for example EP 115627 (Armour), GB 2,127,689 (Sandoz), US 4,548,922 (Carey *et al*) and Hirai *et al.*, *Int. J. Pharm.*, 2, 165-184, 1981. However, a recognised problem with surfactant absorption promoters is that they can cause irritation and histolesion at the site of administration. These problems become of great importance when the peptide is administered regularly over a prolonged period.

The present applicants have previously found that glycyrrhizinic acid and its salts are excellent absorption promoters for calcitonins and do not give rise to the above-mentioned problems of local toxicity and irritation. Compositions comprising a calcitonin and a glycyrrhizinate are described in our EPA 327756, which includes both liquid and solid formulations. Liquid formulations conventionally contain a preservative and EPA 327756 refers to the use of

alkyl p-hydroxybenzoates (parabens) such as methyl and propyl p-hydroxybenzoate as suitable preservatives.

However, it has subsequently been shown that the
5 antibacterial and preservative actions of the parabens are reduced by the glycyrrhizinate component of the formulation. In addition it would be desirable to increase the absorption of calcitonins still further.

10 We have now surprisingly found that the inclusion of benzyl alcohol in a composition comprising a calcitonin and a glycyrrhizinate not only gives rise to a preservative action which is not diminished by the glycyrrhizinate, but also enhances the absorption of the calcitonin in a synergistic
15 manner. Thus the use of a glycyrrhizinate in combination with benzyl alcohol increases the transmucosal absorption of a calcitonin by more than the sum of the respective effects of benzyl alcohol and glycyrrhizinate alone.

20 In a first aspect, therefore, the present invention provides pharmaceutical compositions comprising a calcitonin; an effective amount of an absorption enhancer which is a glycyrrhizinate; an effective amount of benzyl alcohol and a pharmaceutically acceptable carrier.

25 The present invention also provides a method of enhancing the absorption of a calcitonin across a mucosal membrane, which method comprises co-administering with the calcitonin an effective amount of an absorption enhancer which is a
30 glycyrrhizinate, and an effective amount of benzyl alcohol.

Whilst preservatives are generally only used in liquid formulations, absorption enhancers are required in both liquid and solid formulations of calcitonins, and hence the
35 present invention includes within its scope both solid and liquid compositions.

The term glycyrrhizinate as used herein is intended to mean both glycyrrhizinic acid and its carboxylate salts. Particular glycyrrhizinate salts are ammonium glycyrrhizinate and the alkali metal salts e.g. sodium glycyrrhizinate and potassium glycyrrhizinate. A preferred salt is ammonium glycyrrhizinate.

The term calcitonin as used herein is intended to refer to that class of pharmacologically active polypeptides including not only naturally occurring calcitonins but also various derivatives and analogues thereof, e.g. in which one or more of the amino acid residues or sequences naturally present is omitted, replaced, reversed or otherwise derivatised or in which the N- or C-terminal is modified.

The general term calcitonin, as used hereinafter, is intended to mean all such calcitonins whether naturally occurring or synthetic.

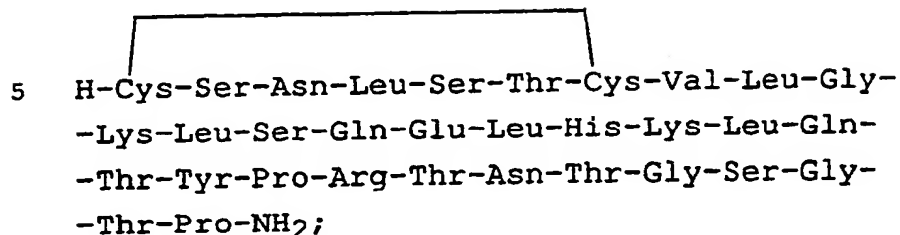
Examples of naturally occurring calcitonins include: human calcitonin, Chemical Abstract Service Registry Number (CAS RN) = 21215-62-3, which has the structure:

H-Cys-Gly-Asn-Leu-Ser-Thr-Cys-Met-Leu-Gly-
-Thr-Tyr-Thr-Gln-Asp-Phe-Asn-Lys-Phe-His-
-Thr-Phe-Pro-Gln-Thr-Ala-Ile-Gly-Val-Gly-
-Ala-Pro-NH₂;

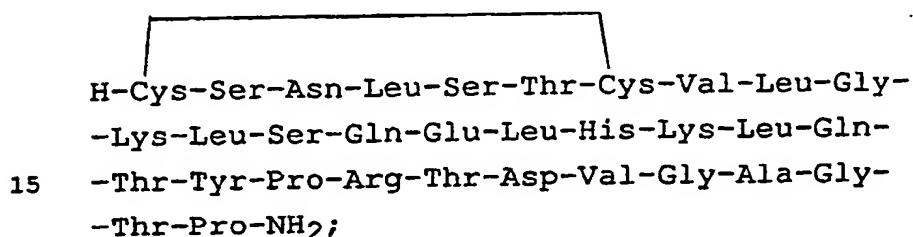
rat calcitonin (CAS RN = 11118-25-5) which has the structure:

H-Cys-Gly-Asn-Leu-Ser-Thr-Cys-Met-Leu-Gly-
-Thr-Tyr-Thr-Gln-Asp-Leu-Asn-Lys-Phe-His-
-Thr-Phe-Pro-Gln-Thr-Ser-Ile-Gly-Val-Gly-
-Ala-Pro-NH₂;

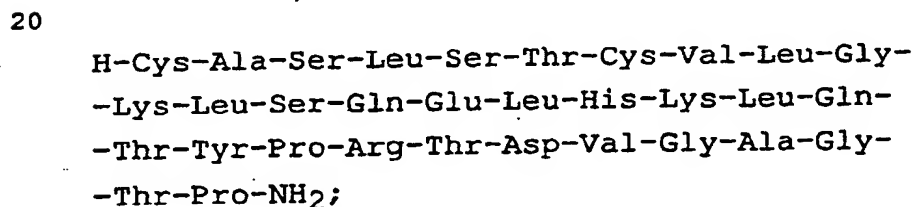
salmon calcitonin (CAS RN = 47931-85-1) which has the structure:



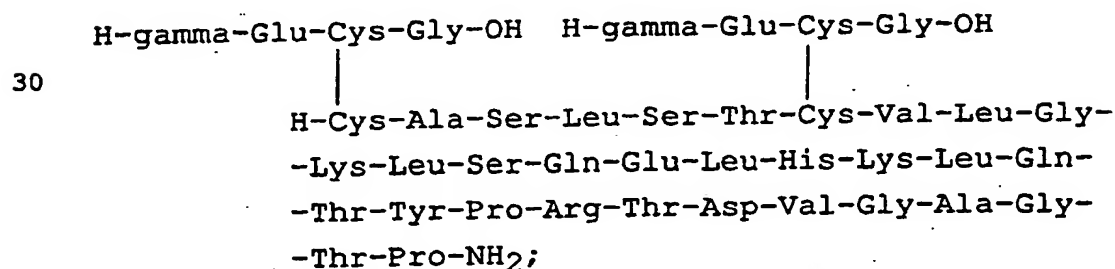
10 eel calcitonin (CAS RN = 57014-02-5) which has the structure:



reduced chicken calcitonin I (CAS RN = 96157-98-1) which has the structure:



25 chicken calcitonin II (CAS RN = 103468-65-1) which has the structure:



35 ox calcitonin (CAS RN = 26112-29-8) which has the structure:

10 H-Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-Ser-
-Ala-Tyr-Trp-Lys-Asp-Leu-Asn-Asn-Tyr-His-
5 -Arg-Phe-Ser-Gly-Met-Gly-Phe-Gly-Pro-Glu-
-Thr-Pro-NH₂;

pig calcitonin (CAS RN = 12321-44-7) which has the structure:

10 H-Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-Ser-
-Ala-Tyr-Trp-Arg-Asn-Leu-Asn-Asn-Phe-His-
-Arg-Phe-Ser-Gly-Met-Gly-Phe-Gly-Pro-Glu-
-Thr-Pro-NH₂; and

15 sheep calcitonin (CAS RN = 40988-57-6) which has the
structure:


20 H-Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-Ser-
-Ala-Tyr-Trp-Lys-Asp-Leu-Asn-Asn-Tyr-His-
-Arg-Tyr-Ser-Gly-Met-Gly-Phe-Gly-Pro-Glu-
-Thr-Pro-NH₂.

25 Examples of calcitonins wherein one or more amino acids
have been omitted are the des-[Ser², Tyr²²]-Gly⁸-calcitonins
described in US 4,597,900 and the des-[Tyr²²]-salmon
calcitonin described in US 4,304,692.

30 Examples of calcitonins wherein the naturally occurring
sequence has been modified include the 1,7-dicarba-
calcitonins such as eel 1,7-dicarbacalcitonin (elcatonin CAS
RN = 60731-46-6) which has the structure:

35 (CH₂)₅
CO-Ser-Asn-Leu-Ser-Thr-NH-CH-CO-Val-Leu-Gly-
-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-
-Thr-Tyr-Pro-Arg-Thr-Asp-Val-Gly-Ala-Gly-
40 -Thr-Pro-NH₂;

salmon 1,7-dicarbacalcitonin (CAS RN = 60864-37-1) which has the structure:

5  (CH₂)₅
CO-Ser-Asn-Leu-Ser-Thr-NH-CH-CO-Val-Leu-Gly-
-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-
-Thr-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Gly-
10 -Thr-Pro-NH₂; and

human 1,7-dicarbacalcitonin (CAS RN = 66811-56-1) which has the structure:

15 (CH₂)₅
 $\left[\begin{array}{c} \text{CO-Gly-Asn-Leu-Ser-Thr-NH-CH-CO-Met-Leu-Gly-} \\ \text{-Thr-Tyr-Thr-Gln-Asp-Phe-Asn-Lys-Phe-His-} \\ \text{-Thr-Phe-Pro-Gln-Thr-Ala-Ile-Gly-Val-Gly-} \\ \text{-Ala-Pro-NH}_2. \end{array} \right]_5$
20

In the context of the present invention, a particularly preferred calcitonin is elcatonin (CAS RN = 60731-46- 6). The preparation and properties of elcatonin and related 1,7-dicarbacalcitonins are described in British Patent Number 1,516,947 (Toyo Jozo).

Another preferred calcitonin is naturally occurring eel calcitonin (CAS RN = 57014-02-5). The preparation and properties of eel calcitonin are described in US 3,988,309 (Matsuda *et al*).

The compositions of the present invention suitably can be administered by methods known in the art for transmucosal and transdermal delivery of pharmacologically active substances. The compositions can be administered to, for example, the nasal, sublingual, buccal, rectal, vaginal and colonic mucosa and to the skin. They can take the form of drops, aerosols,

tablets, capsules, powders, gels, ointments, inserts, suppositories, pessaries, patches and membranes. The compositions can also take the form of enterically coated solid oral compositions as described in, for example, EP 127535 (Hadassah Medical Organisation). The compositions for sublingual and buccal administration can also take the form of wafers as described in PCT/GB91/00651. Such wafers are formed substantially from starch, and suitably have a thickness of from 0.3 to 1.0 mm.

Particular compositions are those intended for administration to the nasal, buccal, sublingual, rectal and vaginal mucosa.

When the composition is intended for delivery to the nasal mucosa, particular dosage forms are solutions, aerosols, drops, gels and powders.

Particular dosage forms for buccal and sublingual administration are gels, suspensions, tablets, patches, powders, ointments, solutions, aerosols and wafers.

Aerosol formulations typically comprise a solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and are usually presented in single or multidose quantities in a sealed container. The sealed container can take the form of a cartridge or refill for use with an atomising device, or it can take the form of a unitary dispensing device such as a single dose nasal inhaler (see French Patent Application FR 2578426) or an aerosol dispenser fitted with a metering valve and which is intended for disposal once the contents of the container have been exhausted. Where the dosage form comprises an aerosol dispenser, it will contain a propellant which can be a compressed gas such as compressed air or an organic propellant such as a fluorochlorohydrocarbon. Such aerosol dispensers are well known in the art. The aerosol dosage forms can also take the form of a pump-atomiser and such forms are also well known in the art. The atomising or

dispensing devices for dispensing aerosol sprays typically are designed to dispense particles of a size greater than 10 micrometres. In order to ensure that significant quantities of the composition remain in contact with the oral or nasal mucosa, and are not inhaled, the particles suitably are approximately 10-160 micrometres in size.

When the composition is intended to be administered as a liquid spray, the viscosity of the liquid composition can be adjusted as necessary according to known methods to ensure that the composition is sprayable.

When the composition is intended for application to the rectal and vaginal mucosa particular dosage forms include pessaries, suppositories, solutions, foams, suspensions, gels, ointments, tablets and soft gelatin capsules.

Compositions for rectal or vaginal administration are generally presented as a solid suppository or a semisolid or liquid formulation filled into a soft gelatin capsule. It will be appreciated therefore that the excipients for use in such suppository or capsule formulations will be selected and if necessary admixed to give a formulation of the desired consistency at room and body temperatures. Thus, the suppository base or carrier may for example comprise one or more components selected from an oil, a fat, a polyglycolysed glyceride and a polyethylene glycol. The oil and/or fat preferably comprises one or more triglycerides as the main component, such as coconut oil, fractionated coconut oil (e.g. Miglyol) palm kernel oil, palm fat, cocoa butter or lard. Examples of hard fat suppository bases include Witepsol and Suppocire. A saturated or unsaturated polyglycolysed glyceride may be for example a saturated polyglycolysed glyceride consisting of C₈-18 glycerides and polyethylene glycol esters such as are available under the trade name Gelucire e.g. Gelucire 35/10, 37/02 or 44/14; a saturated polyglycolysed C₈-C₁₀ glyceride such as that available under the trade name Labrasol; or an unsaturated

polyglycolysed glyceride consisting of C₁₆-C₂₀ glycerides and polyethylene glycol esters such as those available under the trade name Labrafils e.g. Labrafil WL 2609 BS or M 2125 CS. For use in a capsule formulation the polyethylene glycol component is preferably liquid at room temperature such as polyethylene glycol-200, 300, 400 or 600, whereas for a solid suppository a polyethylene glycol of higher molecular weight is preferred. The relative proportions of the excipients will of course depend inter alia on the consistency of the formulation required.

Compositions containing a polyglycolysed glyceride optionally with a polyethylene glycol are preferred. Such compositions can also be adapted for oral administration e.g. in hard or soft gelatin capsules, which are preferably enterically coated.

When the composition is enterically coated and is intended for oral administration, typically it can take the form of a tablet or capsule coated with a coating agent which ensures passage of the calcitonin through the stomach and its subsequent release preferably in the colon. Suitable coating agents include anionic polymers such as acrylic acid/methacrylic acid ester copolymers (e.g. Eudragit S).

The solvents or liquid carriers used in the present formulations are preferably aqueous but can also be chosen from the physiologically acceptable non-aqueous solvents. Examples of non-aqueous solvents or carriers are alcohols, particularly polyhydroxy alcohols such as propylene glycol and glycerol, and vegetable and mineral oils. Such non-aqueous solvents or carriers can be added in various concentrations to water to form solutions, oil-in-water emulsions and water-in-oil emulsions. The solvent preferably is water.

In addition to a solvent or carrier, the liquid formulations of the present invention can contain excipients such as

antioxidants, stabilisers, preservatives, agents for adjusting viscosity (e.g. Carbapol, Keltrol or cellulose derivatives), agents for adjusting tonicity (e.g. sodium chloride, glycine or mannitol), and buffering agents. If
5 desired a further preservative eg. parabens may be used in addition to benzyl alcohol, but in general this is not necessary.

The compositions can also contain a protease inhibitor,
10 preferably a non-surfactant protease inhibitor, for example as described in EP 127535.

In general, the above-mentioned compositions can be made according to well known pharmaceutical procedures, see for
15 example Remington's Pharmaceutical Sciences, 17th Edition, Mack Publishing Company, 1985. Soft gelatin capsules may be prepared for example as described in WO 84/03417 or EPA 122463. Wafer formulations may be prepared for example
as described in PCT/GB91/00651. Thus for example the active
20 ingredient may be incorporated into the wafer mix prior to forming the wafer, or applied to the wafer in the form of a layer or a spray.

The compositions of the present invention can be used in the
25 treatment of diseases such as Paget's disease (osteitis deformans), osteoporosis, including post-menopausal osteoporosis; Sudeck's disease and various hypercalcaemic conditions (see, for example, the Physician's Desk Reference, 42nd Edition, 1988, pages 1796 and 1797).

30

The compositions will be administered to the patient in dosages which contain an amount of calcitonin effective to treat the disease in question.

35 The quantity of pharmacologically active substance in a unit dose of the compositions of the present invention will vary according to the potency of the calcitonin and the nature of the composition. However, in general, a unit dose of a

composition intended for human use typically contains between 1 and 400 International Units (I.U.) of a calcitonin. For elcatonin, a unit dose preferably contains from 5 to 200 I.U. A typical dosage regimen for elcatonin is from 5 to 200 I.U. per day which may be administered in a single dose or in divided doses, for example on consecutive or on alternate days.

The term "International Unit" refers to the appropriate International Reference Preparation (I.R.P.) of human, salmon or porcine calcitonins, or elcatonin, established by the National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire, EN6 3QG, United Kingdom.

When the formulation is a liquid formulation, particularly a spray, the volume of a unit dose typically is in the range 50 to 130 mcl.

The pH of the compositions of the present invention can vary within a broad range according to the chemicophysical properties of the different ingredients in the compositions. However, suitably the pH of the composition is in the range from pH 3 to 8, preferably from approximately pH 4 to approximately pH 7. In order to regulate the pH and maintain a suitable value, a buffering agent may be included in the composition. Examples of buffering agents which may be used include citrates, for example a mixture of citric acid and sodium citrate, acetates and phosphates. In addition to a buffering agent such as those described hereinabove, an alkali metal hydroxide e.g. sodium hydroxide may be incorporated to regulate the pH.

The concentration of the benzyl alcohol is between 0.1 and 5.0% (w/w) of the total weight of the composition. In a liquid or gel composition the benzyl alcohol is suitably present in an amount corresponding to between 0.5 g and 4 g per 100 ml of composition. Preferably the benzyl alcohol is

present in an amount corresponding to approximately 2 g/100 ml. In suppositories, tablets and soft gelatin capsules for rectal or vaginal administration the benzyl alcohol is suitably present in an amount corresponding to between 0.1 g and 1 g per 100 g of composition. Preferably the benzyl alcohol is present in an amount corresponding to between 0.1 g and 0.5 g per 100 g.

The concentration of the glycyrrhizinate absorption enhancer typically is at least 0.1% (w/w), suitably 0.1 to 10% (w/w), and preferably 0.2 to 5% (w/w) of the total weight of the composition.

Where the composition is a liquid or gel composition, the glycyrrhizinate suitably is present in an amount corresponding to between 0.5g and 5g per 100 ml of composition. Preferably the glycyrrhizinate is present in an amount corresponding to approximately 2g/100 ml. In suppositories, tablets and soft gelatin capsules for rectal or vaginal administration the glycyrrhizinate is suitably present in an amount corresponding to between 0.1 g and 2 g per 100 g of composition. Preferably the glycyrrhizinate is present in an amount corresponding to between 0.2 g and 1 g per 100 g.

For aqueous compositions, the final pharmaceutical form, i.e. liquid solution or gel, can also depend upon the pH, the ionic strength of the solution and the concentration of glycyrrhizinate. In general, compositions having a pH of about 5.5 and above will exist as liquids whilst compositions having a lower pH value will tend to be more viscous and, at around pH 4.5, will exist in a gel form.

The invention will now be illustrated in greater detail by the following examples.

Formulations for nasal, sublingual, buccal, rectal or
vaginal administration

Examples 1-3

5

Table 1

	Example No.		
	1	2	3
Elcatonin (mcg)	300	300	300
(6500 I.U./mg potency)			
Ammonium glycyrrhizinate (g)	2	2	2
Citric acid (mg)	37	37	37
Sodium citrate dihydrate	463	463	463
Sodium chloride (mg)	600	600	600
Benzyl alcohol (g)	05	1	2
Distilled water	q.s. to 100 ml		
1N NaOH	q.s. to pH 6		

10

The formulations of Examples 1 to 3 were prepared by mixing together the ammonium glycyrrhizinate, citric acid, sodium citrate dihydrate, sodium chloride, distilled water and sodium hydroxide in a water bath regulated at a temperature of about 70°C. To the resulting solution cooled to room temperature, benzyl alcohol and elcatonin were then added.

15

Examples 4-10

The following compositions were prepared according to the
 5 method described in Examples 1 to 3.

Table 2

	Example No.						
	4	5	6	7	8	9	10
Elcatonin (mcg) (6500 I.U./mg potency)	3690	3690	3690	7380	7380	7380	7380
Ammonium glycyrrhizinate (g)	0.5	2	5	0.5	1	2	5
Citric acid (mg)	37	37	37	37	37	37	37
Sodium citrate dihydrate (mg)	463	463	463	463	463	463	463
Sodium chloride (mg)	600	600	600	600	600	600	600
Benzyl alcohol (g)	2	2	2	2	2	2	2
Distilled water				q.s. to 100 ml			
1N NaOH				q.s. to pH 6			

Examples 11-15

The following compositions were prepared according to the
5 method described in Examples 1 to 3.

Table 3

	Example No.				
	11	12	13	14	15
Elcatonin (mcg)	3690	3690	7380	7380	7380
(6500 I.U./mg potency)					
Ammonium glycyrrhizinate (g)	2	2	2	2	2
Citric acid (mg)	37	37	37	37	37
Sodium citrate dihydrate	463	463	463	463	463
(mg)					
Sodium chloride (mg)	600	600	600	600	600
Benzyl alcohol (g)	0.5	4	0.5	1	4
Distilled water		q.s. to 100 ml			
1N NaOH		q.s. to pH 6			

Examples 16-19

Table 4

5

	Example No.			
	16	17	18	19
Elcatonin (mcg)	3690	3690	7380	7380
(6500 I.U./mg potency)				
Ammonium glycyrrhizinate (g)	2	2	2	2
Citric acid (mg)	37	37	37	37
Sodium citrate dihydrate (mg)	463	463	463	463
Sodium chloride (mg)	600	600	600	600
Benzyl alcohol (g)	0.5	1	0.5	1
Methyl p-hydroxybenzoate (mg)	130	130	130	130
Propyl p-hydroxybenzoate (mg)	20	20	20	20
Distilled water	q.s. to 100 ml			
1N NaOH	q.s. to pH 6			

The formulations of Examples 16 to 19 were prepared by mixing together the ammonium glycyrrhizinate, citric acid, sodium citrate dihydrate, sodium chloride, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, distilled water and sodium hydroxide in a water bath regulated at a temperature of about 70°C. To the resulting solution cooled to room temperature, benzyl alcohol and elcatonin were then added.

Examples 20-21

The following compositions were prepared according to the
5 method described in Examples 1 to 3

Table 5

	Example No.	
	20	21
Elcatonin (mcg)	3690	7380
(6500 I.U./mg potency)		
Ammonium glycyrrhizinate (g)	2	2
Citric acid (mg)	37	37
Sodium citrate dihydrate (mg)	463	463
Sodium chloride (mg)	600	600
Benzyl alcohol (g)	2	2
Distilled water	q.s. to 100 ml	
0.1N NaOH	q.s. to pH 4.5	

10

The formulations of Examples 20 and 21 are gels.

Example 22

Elcatonin (mcg)	7380
(6500 I.U./mg potency)	
Ammonium glycyrrhizinate (g)	2
Acetic acid (mg)	200
Sodium acetate trihydrate (mg)	200
Sodium chloride (mg)	600
Benzyl alcohol (g)	2
Distilled water q.s. to ml	100
1N NaOH q.s. to pH	5.3

5

The formulation of Example 22 was prepared by mixing together the ammonium glycyrrhizinate, acetic acid, sodium acetate trihydrate, sodium chloride, distilled water and sodium hydroxide in a water bath regulated at a temperature of about 70°C. To the resulting solution cooled to room temperature, benzyl alcohol and elcatonin were then added.

TRIAL A

The preparation reported in Example 3 containing ammonium glycyrrhizinate 2% and benzyl alcohol 2% as absorption enhancers, was compared, in a test of pharmacodynamic activity in rats i.e. lowering of calcium concentration in the serum, with the following preparations:

a formulation with the same composition as Example 3 with the exception of benzyl alcohol (reference preparation A); a formulation with the same composition as Example 3 with the exception of ammonium glycyrrhizinate (reference preparation B); a formulation with the same composition as Example 3 with the exception of both benzyl alcohol and ammonium glycyrrhizinate (reference preparation C).

The preparations were administered intranasally with a small catheter, in the volume of 100 μ l/kg body weight (corresponding to 2 I.U./kg), to groups of 10 Sprague Dawley rats weighing 160 ± 20 g. The animals, fasted overnight, were anaesthetized with tribromoethanol (TBE) 2% (9 ml/kg body weight, given i.p.) 15 min before receiving elcatonin.

Serum calcium concentration was measured (with an atomic absorption spectrophotometer VARIAN 30/40) on blood samples obtained in each animal, from the caudal vein, 0, 30, 60, 120 and 180 min after administration of the products.

The results, expressed as residual percentage of serum calcium concentration as compared with baseline values (0 time), are reported in Table 6.

In order to evaluate the relative efficacy of the preparations, the AUC (0-180 min) values were calculated; being AUC calculated on the residual serum calcium, a lower AUC is indicative of a greater pharmacodynamic effect. The AUC (0-180 min) values and the differences (Δ AUC) between the test preparations and reference preparations A and B in

comparison with that of the reference preparation C, which does not contain either ammonium glycyrrhizinate or benzyl alcohol, are reported in Table 7.

- 5 The results obtained show a clear effect of synergism due to the combination of ammonium glycyrrhizinate and benzyl alcohol.

10 **Table 6**

Residual percentage of serum calcium
as compared with baseline values

	0	30 min	60 min	120 min	180 min
Preparation of this invention reported in Example 3	100.0	83.0	73.7	78.7	88.0
Reference preparation A	100.0	85.0	76.1	94.7	89.0
Reference preparation B	100.0	89.6	90.5	92.0	89.5
Reference preparation C	100.0	101.4	93.5	91.7	91.7

Table 7

AUC (0-180 min) calculated on the residual serum calcium		
	AUC	Δ AUC
Preparation of this invention reported in Example 3	14,751	2,318
Reference preparation A	15,884	1,185
Reference preparation B	16,534	535
Reference preparation C	17,069	

TRIAL B

5 The preparation reported in Example 2 containing ammonium glycyrrhizinate 2% and benzyl alcohol 1% as absorption enhancers, was compared, in a test of pharmacodynamic activity in rats i.e. lowering of calcium concentration in the serum, with the following preparations:

- 10 a formulation with the same composition as Example 2 with the exception of benzyl alcohol (reference preparation A); a formulation with the same composition as Example 2 with the exception of ammonium glycyrrhizinate (reference preparation B); a formulation with the same composition as Example 2 with
15 the exception of both benzyl alcohol and ammonium glycyrrhizinate (reference preparation C).

The testing methodologies were the same described for Trial A.

20

The results of residual percentage of serum calcium are reported in Table 8. The AUC and Δ AUC values are reported in Table 9.

- 25 The results obtained show a clear effect of synergism due to the combination of ammonium glycyrrhizinate and benzyl alcohol.

Table 8

Residual percentage of serum calcium
as compared with baseline values

	0	30 min	60 min	120 min	180 min
Preparation of this invention reported in Example 2	100.0	80.2	75.4	77.3	85.0
Reference preparation A	100.0	81.4	79.6	89.8	86.1
Reference preparation B	100.0	87.6	92.0	87.6	85.8
Reference preparation C	100.0	96.8	87.7	90.4	90.4

Table 9

5

AUC (0-180 min) calculated on
the residual serum calcium

	AUC	Δ AUC
Preparation of this invention reported in Example 2	14,631	1,959
Reference preparation A	15,535	1,055
Reference preparation B	16,090	500
Reference preparation C	16,590	

Examples 23 and 24**Table 10**

5

	Example No.	
	23	24
Salmon calcitonin (mcg) (5500 I.U./mg potency)	9090	18180
Ammonium glycyrrhizinate (g)	2	2
Citric acid (mg)	37	37
Sodium citrate dihydrate (mg)	463	463
Benzyl alcohol (g)	2	2
Sodium chloride (mg)	600	600
Distilled water	q.s. to 100 ml	
1N NaOH	q.s. to pH 6	

The formulations of Examples 23 and 24 are prepared by mixing together the ammonium glycyrrhizinate, citric acid, sodium
10 citrate dihydrate, sodium chloride, distilled water and sodium hydroxide in a water bath regulated at a temperature of about 70°C. To the resulting solution cooled to room temperature, benzyl alcohol and salmon calcitonin are then added.

15

Examples 25 and 26

Table 11

5

	Example No.	
	25	26
	10000	20000
Eel calcitonin (mcg) (5000 I.U./mg potency)		
Ammonium glycyrrhizinate (g)	2	2
Citric acid (mg)	37	37
Sodium citrate dihydrate (mg)	463	463
Benzyl alcohol (g)	2	2
Sodium chloride (mg)	600	600
Distilled water	q.s. to 100 ml	
1N NaOH	q.s. to pH 6	

10 The formulations of Examples 25 and 26 are prepared by mixing together the ammonium glycyrrhizinate, citric acid, sodium citrate dihydrate, sodium chloride, distilled water and sodium hydroxide in a water bath regulated at a temperature of about 70°C. To the resulting solution cooled to room temperature, benzyl alcohol and eel calcitonin are then added.

15

Examples 27 and 28

Table 12

5

	Example No.	
	27	28
Chicken calcitonin II (mcg) (5000 I.U./mg potency)	10000	20000
Ammonium glycyrrhizinate (g)	2	2
Citric acid (mg)	37	37
Sodium citrate dihydrate (mg)	463	463
Benzyl alcohol (g)	2	2
Sodium chloride (mg)	600	600
Distilled water	q.s. to 100 ml	
1N NaOH	q.s. to pH 6	

The formulations of Examples 27 and 28 are prepared by mixing together the ammonium glycyrrhizinate, citric acid, sodium
10 citrate dihydrate, sodium chloride, distilled water and sodium hydroxide in a water bath regulated at a temperature of about 70°C. To the resulting solution cooled to room temperature, benzyl alcohol and chicken calcitonin II are then added.

15

Examples 29 and 30

Table 13

5

	Example No.	
	29	30
Human calcitonin (mg)	250	500
(200 I.U./mg potency)		
Ammonium glycyrrhizinate (g)	2	2
Citric acid (mg)	37	37
Sodium citrate dihydrate (mg)	463	463
Benzyl alcohol (g)	2	2
Sodium chloride (mg)	600	600
Distilled water	q.s. to 100 ml	
1N NaOH	q.s. to pH 6	

10 The formulations of Examples 29 and 30 are prepared by mixing together the ammonium glycyrrhizinate, citric acid, sodium citrate dihydrate, sodium chloride, distilled water and sodium hydroxide in a water bath regulated at a temperature of about 70°C. To the resulting solution cooled to room temperature, benzyl alcohol and human calcitonin are then added.

15

Examples 31 and 32

Table 14

5

	Example No.	
	31	32
Pig calcitonin (mg) (60 I.U./mg potency)	834	1668
Ammonium glycyrrhizinate (g)	2	2
Citric acid (mg)	37	37
Sodium citrate dihydrate (mg)	463	463
Benzyl alcohol (g)	2	2
Sodium chloride (mg)	600	600
Distilled water	q.s. to 100 ml	
1N NaOH	q.s. to pH 6	

The formulations of Examples 31 and 32 are prepared by mixing together the ammonium glycyrrhizinate, citric acid, sodium
10 citrate dihydrate, sodium chloride, distilled water and sodium hydroxide in a water bath regulated at a temperature of about 70°C. To the resulting solution cooled to room temperature, benzyl alcohol and pig calcitonin are then added.

15

Powder for nasal administration

Examples 33 and 34

5

Table 15

	Example No.	
	33	34
Elcatonin (mg) (6500 I.U./mg potency)	3.69	7.38
Ammonium glycyrrhizinate (g)	2.0	2.0
Benzyl alcohol (g)	2.0	2.0
Lactose q.s. to (g)	25.0	25.0

- 10 The formulations of Examples 33 and 34 are prepared by wetting the lactose with, benzyl alcohol and with an aqueous solution of elcatonin and drying under vacuum. The dried powder is mixed with ammonium glycyrrhizinate and the final mixture is placed into hard gelatine capsules (25 mg each capsule).
- 15

The powder is administered, after having pierced the capsules, using a nasal insufflator.

Sublingual tablets

Examples 35 and 36

5

Table 16

	Example No.	
	35	36
Elcatonin (mg)	7.7	15.4
(6500 I.U./mg potency)		
Ammonium glycyrrhizinate (g)	4.0	4.0
Benzyl alcohol (g)	4.0	4.0
Sucrose (g)	35.0	35.0
Mannitol (g)	35.0	35.0
Polyethylene glycol 6000 (g)	10.0	10.0
Lactose q.s. to (g)	120.0	120.0

- 10 The formulations of Examples 35 and 36 are prepared by mixing together the sucrose, the mannitol and the lactose. The resulting mixture is wetted with benzyl alcohol and with an aqueous solution of elcatonin, granulated through a stainless steel screen and dried under vacuum. The dried granules are
- 15 mixed with polyethylene glycol and ammonium glycyrrhizinate and then compressed into tablets of 120 mg each.

Buccal tablets

Examples 37 and 38

5

Table 17

	Example No.	
	37	38
Elcatonin (mg)	7.7	15.4
(6500 I.U./mg potency)		
Ammonium glycyrrhizinate (g)	4.0	4.0
Benzyl alcohol (g)	4.0	4.0
Sucrose (g)	30.0	30.0
Mannitol (g)	35.0	35.0
Polyethylene glycol 6000 (g)	15.0	15.0
Carbopol 934 (g)	15.0	15.0
Lactose q.s. to (g)	150.0	150.0

- 10 The formulations of Examples 37 and 38 are prepared by mixing together the sucrose, the mannitol and the lactose. The resulting mixture is wetted with benzyl alcohol and with an aqueous solution of elcatonin, granulated through a stainless steel screen and dried under vacuum. The dried granules are
- 15 mixed with ammonium glycyrrhizinate, Carbopol and polyethylene glycol and then compressed into tablets of 150 mg each.

Oral tablets for colonic delivery

Examples 39 and 40

5

Table 18

	Example No.	
	39	40
Elcatonin (mg)	15.4	30.8
(6500 I.U./mg potency)		
Ammonium glycyrrhizinate (g)	6.0	6.0
Benzyl alcohol (g)	4.0	4.0
Pregelatinized starch (g)	80.0	80.0
Magnesium stearate (g)	2.0	2.0
Lactose q.s. to (g)	210.0	210.0
Eudragit S (g)	20.0	20.0
Polyethylene glycol 6000 (g)	2.0	2.0

- 10 The formulations of Examples 39 and 40 are prepared by mixing together the pregelatinized starch and the lactose. The resulting mixture is wetted with benzyl alcohol and with an aqueous solution of elcatonin, granulated through a stainless steel screen and dried under vacuum. The dried granules are
- 15 mixed with ammonium glycyrrhizinate and magnesium stearate and then compressed into tablets of 210 mg each.

The tablets are coated with an aqueous suspension of polyethylene glycol and Eudragit, to a final weight of 232

20 mg/tablet.

Dosage form for vaginal administration

Examples 41 and 42

5

Table 19

	Example No.	
	41	42
Elcatonin (mg) (6500 I.U./mg potency)	15.4	30.8
Ammonium glycyrrhizinate (g)	8.0	8.0
Benzyl alcohol (g)	8.0	8.0
Corn starch (g)	180.0	180.0
Adipic acid (g)	140.0	140.0
Sodium bicarbonate (g)	110.0	110.0
Magnesium stearate (g)	20.0	20.0
Lactose q.s. to (g)	1600.0	1600.0

- 10 The formulations of Examples 41 and 42 are prepared by mixing together the ammonium glycyrrhizinate, the corn starch, the adipic acid and the lactose. The resulting mixture is wetted with benzyl alcohol and with an aqueous solution of elcatonin, granulated through a stainless steel screen and
- 15 dried under vacuum. The dried granules are mixed with sodium bicarbonate and magnesium stearate and then compressed into tablets of 1.6 g each.

Dosage form for rectal administration**Example 43**

5

Elcatonin (mg) (6500 I.U./mg potency)	15.4
Ammonium glycyrrhizinate (g)	6.0
Benzyl alcohol (g)	4.0
Distilled water (g)	100.0
Hard fat q.s. to (g)	1500.0

10 The formulation of Example 43 is prepared by mixing together the ammonium glycyrrhizinate, benzyl alcohol and distilled water in a water bath regulated at a temperature of about 70°C. The solution is cooled to about 40°C, elcatonin is dissolved and then the resulting solution is incorporated into hard fat melted at about 40°C.

15 The final mixture is poured into suppository moulds and cooled to room temperature, thus obtaining suppositories of 1.5 g each.

Formulations for vaginal or rectal administration

Examples 44-46

5

Table 20

	Example No.		
	44	45	46
Elcatonin (mg)	7.4	14.8	29.6
(6500 I.U./mg potency)			
Polyethylene glycol 600 (g)	550.0	550.0	550.0
Gelucire 44/14 (g)	400.0	400.0	400.0
Distilled water (g)	42.1	42.1	42.1
Ammonium glycyrrhizinate (g)	5.0	5.0	5.0
Benzyl alcohol (g)	2.0	2.0	2.0
Sodium chloride (mg)	300.0	300.0	300.0
Sodium citrate dihydrate (mg)	231.5	231.5	231.5
Sodium hydroxide (mg)	350.0	350.0	350.0
Citric acid (mg)	18.5	18.5	18.5

10 The formulations of Examples 44 to 46 were prepared by mixing together the ammonium glycyrrhizinate, citric acid, sodium citrate dihydrate, sodium chloride, sodium hydroxide, distilled water and elcatonin in a water bath regulated at a temperature of about 70°C.

15

The resulting solution, cooled to about 55°C, was incorporated into a mixture of Gelucire, polyethylene glycol and benzyl alcohol heated to about 55°C.

20 The final mixture, cooled to about 30°C, was filled into soft gelatin capsules (1 g each capsule).

Examples 47-49

Table 21

5

	Example No.		
	47	48	49
Elcatonin (mg)	7.4	14.8	29.6
(6500 I.U./mg potency)			
Polyethylene glycol 600 (g)	400.0	400.0	400.0
Witepsol S55 (g)	450.0	450.0	450.0
Miglyol 812 (g)	100.0	100.0	100.0
Distilled water (g)	42.1	42.1	42.1
Ammonium glycyrrhizinate (g)	5.0	5.0	5.0
Benzyl alcohol (g)	2.0	2.0	2.0
Sodium chloride (mg)	300.0	300.0	300.0
Sodium citrate dihydrate (mg)	231.5	231.5	231.5
Sodium hydroxide (mg)	350.0	350.0	350.0
Citric acid (mg)	18.5	18.5	18.5

The formulations of Examples 47 to 49 were prepared by mixing together the ammonium glycyrrhizinate, citric acid, sodium
 10 citrate dihydrate, sodium chloride, sodium hydroxide, distilled water and elcatonin in a water bath regulated at a temperature of about 70°C.

The resulting solution, cooled to about 55°C, was
 15 incorporated into a mixture of Witepsol, Miglyol, polyethylene glycol and benzyl alcohol heated to about 55°C.

The final mixture, cooled to about 30°C, was filled into soft gelatin capsules (1 g each capsule).

20

Dosage form for transdermal administration**Example 50**

5

Elcatonin (mg)	6
(6500 I.U./mg potency)	
Ammonium glycyrrhizinate (g)	2
Benzyl alcohol (g)	2
Carbopol 934 (g)	2
Citric acid (mg)	37
Sodium citrate dihydrate (mg)	463
Sodium chloride (mg)	600
Distilled water	q.s.to 100 g
1N NaOH	q.s. to pH 6

10 The formulation of Example 50 is prepared by mixing together the ammonium glycyrrhizinate, citric acid, sodium citrate dihydrate, sodium chloride, Carbopol 934, sodium hydroxide and part of distilled water in a water bath regulated at a temperature of about 70°C. To the resulting gel, cooled to room temperature, a solution of elcatonin and benzyl alcohol in the remaining part of distilled water, is then added.

15

The final gel is filled into patches of 500 mg each.

Formulations for nasal, sublingual, buccal, rectal or vaginal administration

Examples 51-52

5

Table 22

	Example No.	
	51	52
Elcatonin (mcg) (6500 I.U./mg potency)	3690	7380
Ammonium glycyrrhizinate (g)	2	2
Citric acid (mg)	37	37
Sodium citrate dihydrate (mg)	463	463
Mannitol (g)	3.3	3.3
Benzyl alcohol (g)	2	2
Distilled water	q.s. to 100 ml	
1N NaOH	q.s. to pH 6	

10

The formulations of Examples 51 and 52 were prepared by mixing together the ammonium glycyrrhizinate, citric acid, sodium citrate dihydrate, mannitol, distilled water and sodium hydroxide in a water bath regulated at a temperature of about 70°C. To the resulting solution cooled to room temperature, benzyl alcohol and elcatonin were then added.

15

Examples 53-54

Table 23

5

	Example No.	
	53	54
Elcatonin (mcg) (6500 I.U./mg potency)	3690	7380
Ammonium glycyrrhizinate (g)	2	2
Citric acid (mg)	37	37
Sodium citrate dihydrate (mg)	463	463
Glycine (g)	1.6	1.6
Benzyl alcohol (g)	2	2
Distilled water	q.s. to 100 ml	
1N NaOH	q.s. to pH 6	

10 The formulations of Examples 53 and 54 were prepared by mixing together the ammonium glycyrrhizinate, citric acid, sodium citrate dihydrate, glycine, distilled water and sodium hydroxide in a water bath regulated at a temperature of about 70°C. To the resulting solution cooled to room temperature, benzyl alcohol and elcatonin were then added.

Examples 55 - 60

Table 23

5

	Example No.					
	55	56	57	58	59	60
Elcatonin (mcg) (6500 I.U./mg potency)	14800	29600	14800	29600	14800	29600
Ammonium glycyrrhizinate (g)	2	2	2	2	2	2
Citric acid (mg)	37	37	37	37	37	37
Sodium citrate dihydrate (mg)	463	463	463	463	463	463
Sodium chloride (mg)	600	600	-	-	-	-
mannitol (g)	-	-	3.3	3.3	-	-
glycine (g)	-	-	-	-	1.6	1.6
Benzyl alcohol (g)	2	2	2	2	2	2
Distilled water	q.s. to 100 ml					
1N NaOH	q.s. to pH6					

The compositions are prepared in an analogous manner to Example 1.

CLAIMS:

1. A pharmaceutical composition comprising a calcitonin, an effective amount of an absorption enhancer
5 which is a glycyrrhizinate, an effective amount of benzyl alcohol and a pharmaceutically acceptable carrier.
2. A composition according to claim 1 wherein the glycyrrhizinate is ammonium glycyrrhizinate.
- 10 3. A composition according to either of claims 1 or 2 wherein the glycyrrhizinate is present in a concentration corresponding to at least 0.1% (w/w) of the total weight of the composition.
- 15 4. A composition according to any of claims 1 to 3 wherein the concentration of benzyl alcohol is between 0.1 and 5.0% (w/w).
- 20 5. A composition according to any one of claims 1 to 4 wherein the calcitonin is elcatonin.
- 25 6. A composition according to any one of claims 1 to 5 which additionally contains a polyglycolysed glyceride.
- 30 7. A composition according to any one of claims 1 to 6 in the form of a liquid, gel or semisolid suitable for application to the nasal, buccal, sublingual, rectal or vaginal mucosa.
- 35 8. A composition according to any one of claims 1 to 7 wherein the glycyrrhizinate is present in an amount corresponding to between 0.5 g and 5 g per 100 ml of composition and the benzyl alcohol is present in an amount corresponding to between 0.5 g and 4 g per 100 ml of composition.

9. A liquid pharmaceutical composition comprising, as a carrier, an aqueous solution buffered to approximately pH 6; a non-toxic effective amount of a preservative; and, per 100ml of composition, 20,000-200,000 International Units of elcatonin, approximately 2g of ammonium glycyrrhizinate and approximately 2g of benzyl alcohol.

10. A composition according to any one of claims 1 to 9 which is packaged for administration as a spray.

10

11. A composition according to any one of claims 1 to 6 adapted for rectal or vaginal administration.

12. A composition according to claim 11 wherein the glycyrrhizinate is present in an amount corresponding to between 0.1 g and 2 g per 100 g of composition and the benzyl alcohol is present in an amount corresponding to between 0.1 g and 1 g per 100 g of composition.

13. A composition according to any one of claims 1 to 12 which has a pH in the range from approximately 4 to approximately 7.

14. A pharmaceutical composition containing a calcitonin, benzyl alcohol and glycyrrhizinate substantially as described herein with reference to Examples 1 to 60.

15. A process for preparing a pharmaceutical composition containing a calcitonin, benzyl alcohol and glycyrrhizinate substantially as described herein with reference to Examples 1 to 60.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 92/02321

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)⁶

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K37/30; A61K47/26; A61K47/10; A61K9/00

II. FIELDS SEARCHEDMinimum Documentation Searched⁷

Classification System

Classification Symbols

Int.Cl. 5

A61K

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched⁸**III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹**

Category ⁹	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	EP,A,0 327 756 (ISF SOCIETA PER AZIONI) 16 August 1989 cited in the application see tables 7-10 ---	1-15
A	EP,A,0 371 010 (YAMANOUCHI PHARMACEUTICAL CO. LTD.) 30 May 1990 cited in the application see claim 1; examples 2-4; tables 1-2 ---	1-15
A	EP,A,0 156 565 (YAMANOUCHI PHARMACEUTICAL CO. LTD.) 2 October 1985 see page 5, line 4 - page 6, line 7; example 7 ---	1-15
P,A	EP,A,0 471 618 (TOYO JOZO CO. LTD.) 19 February 1992 see page 4, line 50 - line 55; examples 5-7 ---	1-15

⁹ Special categories of cited documents : ¹⁰

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"A" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
22 DECEMBER 1992	10.02.93
International Searching Authority EUROPEAN PATENT OFFICE	Signature of Authorized Officer FOERSTER W.K.

Form PCT/ISA/210 (second sheet) (January 1989)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

EP 9202321
SA 65600

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 22/12/92

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0327756	16-08-89	GB-A, B 2212062	19-07-89
		BE-A- 1002333	18-12-90
		FR-A- 2623090	19-05-89
EP-A-0371010	30-05-90	EP-A, B 0183527	04-06-86
		JP-A- 61267528	27-11-86
		US-A- 4788221	29-11-88
		US-A- 4690952	01-09-87
EP-A-0156565	02-10-85	JP-A- 60188315	25-09-85
		DE-A- 3583622	05-09-91
		US-A- 4879118	07-11-89
EP-A-0471618	19-02-92	JP-A- 4099729	31-03-92
		AU-A- 8244591	20-02-92
		CA-A- 2047903	17-02-92

EPO FORM P0079

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

This Page blank (uspio)